RELATIONSHIP BETWEEN LH AND TESTICULAR DEVELOPMENT IN PROGESTERONE-IMPLANTED PREPUBERTAL RAM LAMBS[1]

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Summary

The relationship between systemic luteinizing hormone (LH) and testicular development was investigated in Finn and Suffolk ram lambs treated at 2 wk of age with sc progesterone implants for 0, 4, 8 or 12 wk. Serial samples of blood (30-min intervals for 3 h) were collected from five rams·breed^{-1}·treatment^{-1} at 6, 8, 10, 12, 14, 18 and 22 wk of age plus 1 and 2 wk after implant removal. Scrotal circumference was measured at 10, 14, 18 and 22 wk of age. A testicular biopsy was obtained at 14, 18 and 22 wk for microscopic evaluation of testicular development and spermatogenesis. Pulsatile LH releases occurred 60 to 180 min apart in control lambs between 6 and 22 wk of age; LH secretion was not affected by age except for a decrease ($P<.01$) in mean LH at 22 wk. Transient increases in testosterone were found subsequent to LH pulses. Systemic testosterone increased ($P<.01$) progressively with age, was higher ($P<.05$) in Finn than in Suffolk rams at 18 and 22 wk and was correlated positively ($P<.01$) within breeds with seminiferous tubule diameter at 14 wk and with scrotal circumference at 10 and 14 wk. Tubule diameter was larger ($P<.01$) but testes size and weight were smaller ($P<.01$) in Finn than in Suffolk rams. Progesterone implants decreased ($P<.01$) LH secretion at 4, 6, 8, 10 and 12, but not at 14 wk of age; decreased systemic testosterone between 4 and 14 wk; decreased ($P<.01$) seminiferous tubule diameter at 14 wk, which was related inversely to duration of progesterone treatment; and delayed puberty from 18 to 22 or more wk in rams implanted from 2 to 10 or 14 wk of age. Both control and treated rams with elongated spermatids at 18 vs 22 or more wk had increased LH secretion, higher systemic testosterone and larger tubule diameter and testes size at a younger age. These results suggest that rate of sexual maturation in ram lambs is related to level of postnatal LH stimulation and to the prepubertal age when increased LH stimulation occurs.

(Key Words: Ram Lambs, Luteinizing Hormone, Testosterone, Testicular Development, Progesterone.)

Introduction

Comparisons of plasma luteinizing hormone (LH) concentrations among groups of lambs with genetic differences in fecundity indicated that mean LH concentration and incidence of episodic releases of this hormone were higher in the high fecundity groups at 30 to 50 d of age, but lower at older ages (Thimonier et al., 1972; Bindon and Turner, 1974). Carr and Land (1975) found a positive correlation between plasma LH concentration and testis size in ram lambs at about 12 wk of age, with greater LH concentrations and testis size in rams of the prolific breeds. Most studies that have reported a positive relationship between fecundity and postnatal plasma LH concentrations compared low prolificacy breeds to the prolific Finnsheep (Finn) breed, a breed that also reaches puberty at a young age (Carr and Land, 1975; Land, 1978). Therefore, the earlier appearance and disappearance of elevated plasma gonadotropin concentrations in high fecundity breeds may be associated with rate of sexual maturation rather than with level of fecundity. Progesterone suppresses episodic LH release in immature ewe lambs (Foster and Karsch, 1976) and in mature ewes (Goodman and Karsch, 1980) and rams (Bolt, 1971). Thus, treatment of immature ram lambs with progesterone to suppress LH secre-

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tion would provide a model to evaluate the influence of LH secretion on testicular development, testosterone secretion, and puberty without direct stimulation of spermatogenesis from the exogenous steroid as could occur with testosterone (Patanelli, 1975).

The objectives of the present study were: 1) to investigate the relationship between systemic LH and testicular development in pre- and postpubertal ram lambs from two breeds of sheep (Finn vs Suffolk) that sexually mature at different ages and 2) to determine the effect of progesterone suppression of postnatal LH secretion for 0, 4, 8 or 12 wk on sexual development in ram lambs.

Materials and Methods

Equal numbers of Finn and Suffolk ram lambs (40/breed) were assigned randomly at 2 wk of age to one of four treatment groups. Treatments consisted of sc implantation (axillary region) of either 12 empty Silastic capsules for a duration of 6 wk (Group 1), or 12 progesterone-filled Silastic capsules for a duration of 4 (Group 2), 8 (Group 3) or 12 (Group 4) wk. Procedures for construction of the progesterone capsules from Silastic tubing (4.65 mm od x 4 cm) and insertion of the capsules were similar to those described by Karsch et al. (1973). Although age at puberty in rams is influenced by breed, month of birth, nutrition or a combination of environmental factors (Land, 1978), puberty occurs in a majority of rams between approximately 18 and 24 wk of age (Carmon and Green, 1952; Schanbacher et al., 1974). The present experiment utilized ram lambs born between May 12 and 27 in anticipation that puberty would occur during the natural breeding season and minimize the seasonal influence on age at puberty.

Samples of blood (5 ml) for LH, testosterone and progesterone measurements were obtained by jugular venipuncture at 30-min intervals for 3 h (0830 to 1130) at 6, 8, 10, 12, 14, 18 and 22 wk of age ± 1 d. In addition, rams implanted with progesterone were bled at 30-min intervals for 3 h at −1 d and 1, 2, 4, 6 and 8 wk after removal of the progesterone capsules. A single blood sample was collected from each lamb at 4 wk of age. Frequency and duration of blood collections were limited by the small body size and blood volume of the lambs, especially Finns, between 4 and 10 wk of age. Blood was collected into heparinized syringes, refrigerated and centrifuged; plasma was stored at −10 C until assayed.

Plasma LH concentration, expressed as ng of NIH-LH-S18/ml of plasma, was determined in duplicate 200-μl volumes by a double antibody radioimmunoassay (Niswender et al., 1969) with modification described previously (Echternkamp, 1978). The LH antiserum[3] bound 40 to 45% of ^{125}I-labelled purified ovine LH (LER-1056-C2) at a working dilution of 1:35,000; antirabbit gamma globulin was diluted 1:120. Sensitivity of the assay (95% of the cpm in the buffer control tubes) was .5 ng LH/ml of plasma. The intraassay variation for duplicate samples was ⩽10% and the interassay variation for standard plasma samples included in each assay was 10.1%.

Testosterone was quantified by a dextran-coated charcoal radioimmunoassay (Lunstra et al., 1978) utilizing antitestosterone serum S-741-#2[4]. Antibody specificity had been published previously (Abraham et al., 1972). One-hundred microliters of plasma, .4 ml of saline and 50 μl of 1 N NaOH were placed in a vial and extracted twice with 2 ml of diethyl ether. Ether extracts were transferred to 12 x 75 mm tubes, evaporated under N_2 flow and assayed directly without further purification. Sensitivity of the assay was 25 pg/tube of testosterone. The intraassay coefficient of variation for duplicate determinations was ⩽10%. The interassay coefficient of variation for a standard plasma sample included in each assay was 15.5%.

Progesterone was quantified by a dextran-coated charcoal radioimmunoassay (Ford et al., 1979) utilizing antiprogesterone serum S-49#6[4] (Abraham et al., 1971). Plasma (.5 ml) was extracted twice with 2.5 ml of heptane. The heptane extracts were transferred to 13 x 100 mm tubes, evaporated under N_2 flow, resuspended in 1 ml of .1 M PO_4 buffer (pH 7.0) and duplicate 200-μl aliquots were assayed in 12 x 75 mm tubes without further purification. Sensitivity of the assay was 30 pg/tube of progesterone. The intraassay variation for duplicate determinations was ⩽10% and the

[3] Antiserum to bovine LH (DJB 3-12/11) was supplied by Dr. D. J. Bolt, USDA-ARS, Beltsville, MD.

[4] Antisera to testosterone and progesterone were purchased from Dr. G. Abraham, UCLA School of Medicine, Harbor General Hospital, Torrance, CA.

interassay variation for a standard plasma pool containing .98 ng of progesterone/ml was 9.2%.

Subsequent to the 3-h blood collection period at 10, 14, 18 and 22 wk of age, scrotal circumference was measured with a flexible tape around the greatest diameter of the scrotum to determine testes size (Hahn et al., 1969).

A testicular biopsy for measurement of seminiferous tubule diameter and spermatogenesis was obtained from each ram 1 d after the blood collection at 14, 18 and 22 wk of age while subjected to general anaesthesia. General anaesthesia was initiated with Na thiopental and maintained with mixtures of oxygen, nitrous oxide and halothane administered in a closed-circuit with soda lime for removal of CO_2. The biopsy and evaluation procedure (Lunstra and Echternkamp, 1983) consisted of a 2-cm incision through the scrotum and tunica vaginalis to expose the testicular capsule. The testicular capsule was blunt dissected with an iris scissors to produce a 2-mm incision. Caution was exercised to avoid disruption of the vascular plexus within the tunica vasculosa of the testicular capsule. The testicular capsule around the edge of the incision was grasped with small mouse-tooth tissue forceps to anchor the testis while a 10 gauge biopsy needle (90° blunt tip sharpened via an internal bevel) was inserted to a depth of 1 to 2 cm; depth depended upon testis size. The biopsy needle was rotated two to three revolutions and withdrawn. An antibiotic suspension (Albacillin[5]) was infused into the incision site and the scrotum was closed with a single suture. The tissue specimen was sequentially preserved in 2% glutaraldehyde in .075 M cacodylate buffer (pH 7.4) and 1% osmium tetroxide in .075 M cacodylate buffer (pH 7.4); dehydrated in ethanol and propylene oxide and embedded in Epon 812/Araldite 502 resins[6] as described previously (Glauert, 1975). Embedded tissue was sectioned at 2 μm thickness, stained with toluidine-borax mixture and evaluated under light microscopy. Fifty to 100 tubules from two areas of each biopsy were measured to obtain an estimate of tubule diameter. The occurrence of complete spermatogenesis within each seminiferous tubule was confirmed by the presence in the lumenal epithelium of spermatids with condensed elongated nuclei.

[5] Upjohn Co., Kalamazoo, MI.

[6] Polysciences, Inc., Warrington, PA.

The testes were surgically removed from all rams at 22 wk of age. Surgical anaesthesia was administered by the same procedure as described in the biopsy protocol. Testes weight and gross morphology were recorded.

Mean concentration and standard deviation of LH and testosterone were calculated for each ram from the seven blood samples collected during the 3-h period. Group means for each sample period were calculated from the ram means. Basal concentration of LH within each ram for each 3-h period was computed as the mean of the two lowest LH concentrations for the seven plasma samples. By using only the two lowest concentrations within each ram and sample period, inclusion of LH concentrations associated with the ascending, descending or peak portion of an LH pulse into the basal concentrations was avoided in several rams. An increase in systemic LH was defined as a pulsatile release when the increase was at least two within animal standard deviations above the basal LH concentration.

Data were analyzed by split-plot analysis of variance to determine the effects of breed, duration of progesterone treatment, age and their interaction on measured variables. The error mean square to test breed effect was animal within breed. The error mean square to test effects of progesterone treatment and progesterone treatment × breed was animal within breed × progesterone treatment. The residual error mean square was used to test effects of age, breed × age, progesterone treatment × age and breed × progesterone treatment × age. Mean differences were tested by Duncan's multiple range test (Steel and Torrie, 1960). Correlations among plasma concentrations of LH and testosterone, seminiferous tubule diameter, percentage of seminiferous tubules with elongated spermatids and testes weight were calculated within breed and age.

Results

Patterns of LH, Testosterone and Progesterone in Systemic Circulation. Systemic progesterone concentrations (figure 1) resulting from the 12 progesterone implants varied among lambs with a trend for higher concentrations in Finn than in Suffolk rams because of the smaller body size of Finns. There was a trend for progesterone to decrease between 8 and 14 wk of age (figure 1) in Group 4. The decrease in progesterone was not a result of

TABLE 1. CHARACTERISTICS OF LH SECRETION DURING PUBERTAL DEVELOPMENT IN CONTROL AND PROGESTERONE-TREATED RAMS[a]

Variable	Group	Treatment[b]	LH at age: 6 wk	8 wk	10 wk	12 wk	14 wk	18 wk	22 wk
Mean LH concentration, ng/ml									
	1	Control	6.2[f]	10.9[f]	5.1[f]	10.4[f]	8.4	7.2	3.3
	2	4-wk implant[c]	.9[g]	8.8[f]	6.1[f]	8.8[f]	7.9	7.3	5.4
	3	8-wk implant[d]	1.2[g]	.8[g]	1.3[g]	7.3[f]	6.3	5.4	3.5
	4	12-wk implant[e]	1.1[g]	2.5[g]	.8[g]	2.7[g]	5.4	7.3	3.2
No. of LH pulses/3 h									
	1	Control	.9[f]	1.7[f]	1.6[f]	2.1[f]	1.8	1.5	1.3
	2	4-wk implant[c]	.1[g]	1.3[f]	1.9[f]	1.8[f]	1.7	1.7	1.6
	3	8-wk implant[d]	.1[g]	.0[g]	.2[g]	1.8[f]	1.7	2.0	1.1
	4	12-wk implant[e]	.1[g]	.4[g]	.1[g]	.4[g]	1.6	1.9	1.1

[a]Data for the five Finn and Suffolk rams/treatment group were combined. Standard errors for the least-squares means were ± .8 for mean LH concentration and ± .4 for number of LH pulses/3 h.

[b]Rams in Groups 2, 3 and 4 received progesterone implants at 2 wk of age. Control rams received a sham implant.

[c]Implants removed subsequent to blood collection at 6 wk of age.

[d]Implants removed at 10 wk of age.

[e]Implants removed at 14 wk of age.

[f,g]Treatment means differ within age ($P<.01$).

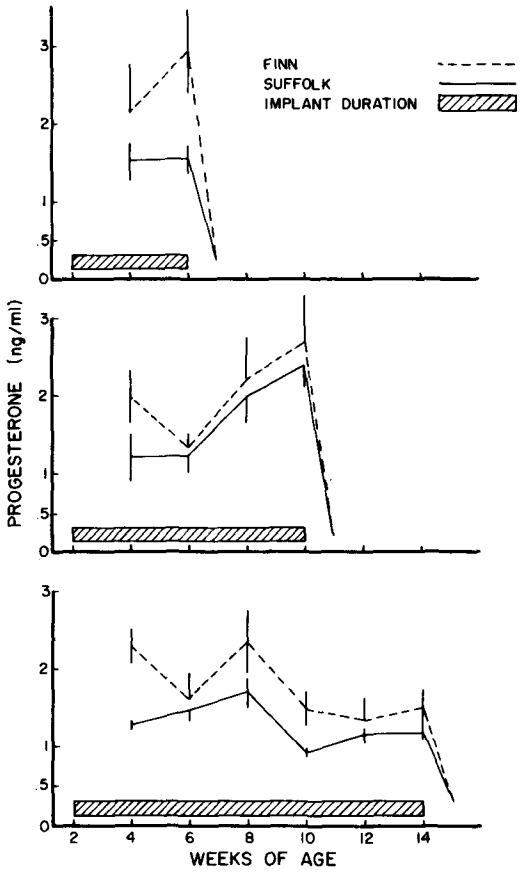

Figure 1. Plasma progesterone concentration in Finn and Suffolk ram lambs implanted with progesterone capsules (horizontal bar) at 2 wk of age for a duration of 4, 8 or 12 wk.

depletion of progesterone from the capsule because significant amounts remained in all capsules after removal.

Ram lambs implanted with progesterone had lower (P<.01) mean plasma concentrations of LH (table 1) at 4, 6, 8, 10 and 12 wk of age, but not (P>.05) at 14 wk (Group 4) relative to control lambs. Removal of the progesterone implants resulted in an increase (P<.05) in mean LH concentration within 1 wk (figure 2). Mean testosterone concentrations were lower (P<.01) in plasma of progesterone-implanted than in control rams while progesterone implants were present.

Mean plasma concentration of testosterone increased (P<.01) progressively with age (figure 2) in the control rams between 4 and 22 wk and in progesterone-treated rams subsequent to implant removal. Both control and progesterone-treated Finn rams had higher (P<.05) mean testosterone concentration at 18 and 22 wk of age than Suffolk rams.

Plasma concentrations of LH and testosterone from 8 through 18 wk of age for Finn and Suffolk rams with (early maturing, n=14) and without (late maturing, n=6) elongated spermatids in the seminiferous tubules at 18 wk of age are illustrated in figure 3. Rams with elongated spermatids had higher basal (P<.10), mean (P<.05) and maximal (P<.05) concentrations

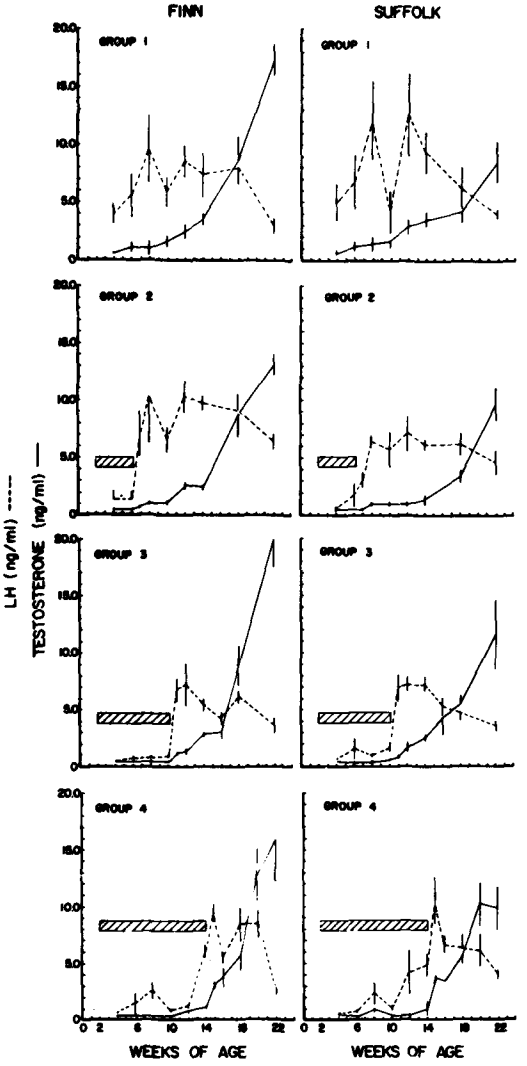

Figure 2. Mean concentrations of LH and testosterone in plasma of Finn (left panels) and Suffolk (right panels) ram lambs treated with progesterone implants (horizontal bar); Group 1 (control), Group 2 (progesterone-implanted, 2 to 6 wk of age), Group 3 (progesterone-implanted, 2 to 10 wk) and Group 4 (progesterone-implanted, 2 to 14 wk).

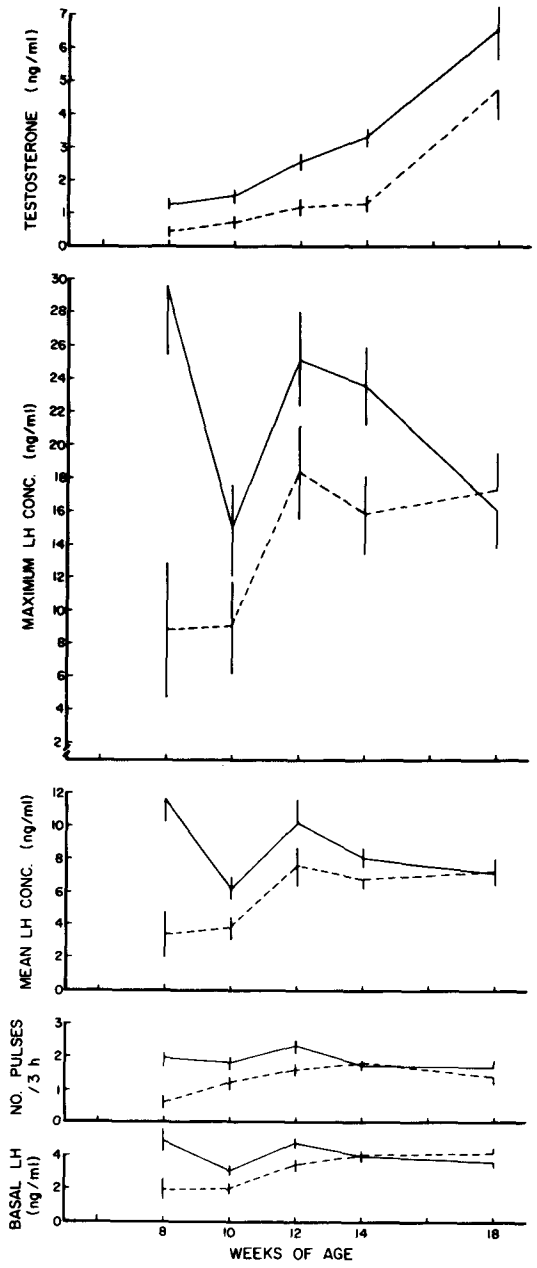

Figure 3. Comparison of mean plasma concentrations of testosterone, maximum LH, average LH, number of pulses/3 h and basal LH between ram lambs in Groups 1 and 2 with (——) or without (- - - -) elongated spermatids in the seminiferous tubules at 18 wk of age.

of LH and number of LH pulses per 3 h (P<.01) at 8 wk of age, and higher (P<.10) basal LH concentration and number of LH pulses at 12 wk of age. Mean testosterone concentration was higher (P<.05) in plasma of early than of late maturing rams throughout the 10-wk period. Mean LH concentration for wk 8 through 14 of age in these rams was correlated positively with mean testosterone concentration (r=.89, P<.01) for the same period. Differences in patterns of systemic LH and testosterone between two Suffolk rams (Group 2) that differed in age at puberty and were representative of rams in the above two groups, are further contrasted in figure 4. Ram 173 (lower panel) had elongated spermatids at 18 wk of age, whereas, elongated spermatids were not found in ram 183 (upper panel) by 22 wk. Low LH and testosterone concentrations were found at 6 wk of age when the progesterone implants were present. After removal of the implants (6 wk of age), LH concentration and pulse frequency and testosterone concentration increased significantly, with the increase being sooner and greater in ram 173, the earlier maturing ram.

Patterns of LH and testosterone in sequential samples of plasma from control Finn and Suffolk ram lambs between 6 and 22 wk of age, or from progesterone-treated ram lambs subsequent to implant removal, indicated the occurrence of pulsatile releases of LH followed frequently by a transient rise in plasma testosterone concentration approximately 30 min later (figure 4). Magnitude and frequency of the LH pulses and, consequently, mean LH concentration in plasma (table 1) varied among control rams irrespective of age except at 22 wk when mean LH concentration was consistently lower (P<.05).

Scrotal Circumference and Testes Weight. Scrotal circumference (table 2) increased with age (P<.01) and was larger for Suffolk than for Finn rams (P<.01). Within Finn rams, scrotal circumference was smaller at 10 wk of age for rams in Groups 3 (P<.05) or 4 (P<.01) than for controls (table 2). Scrotal circumference for Suffolk rams at 10 wk of age did not differ (P>.05) among treatments, but was larger at 14 wk of age for control rams than for rams in Groups 2 (P<.05), 3 (P<.01) or 4 (P<.01). There were no treatment effects (P>.05) at 14, 18 and 22 wk of age for Finn rams, or at 18 and 22 wk for Suffolk rams. Correlations, pooled across treatment, between scrotal circumference and mean testosterone concentration in plasma were r = .67 (P<.01), .58 (P<.01), .27 and .59 (P<.05) at 10, 14, 18 and 22 wk of age, respectively, for Finn rams and r

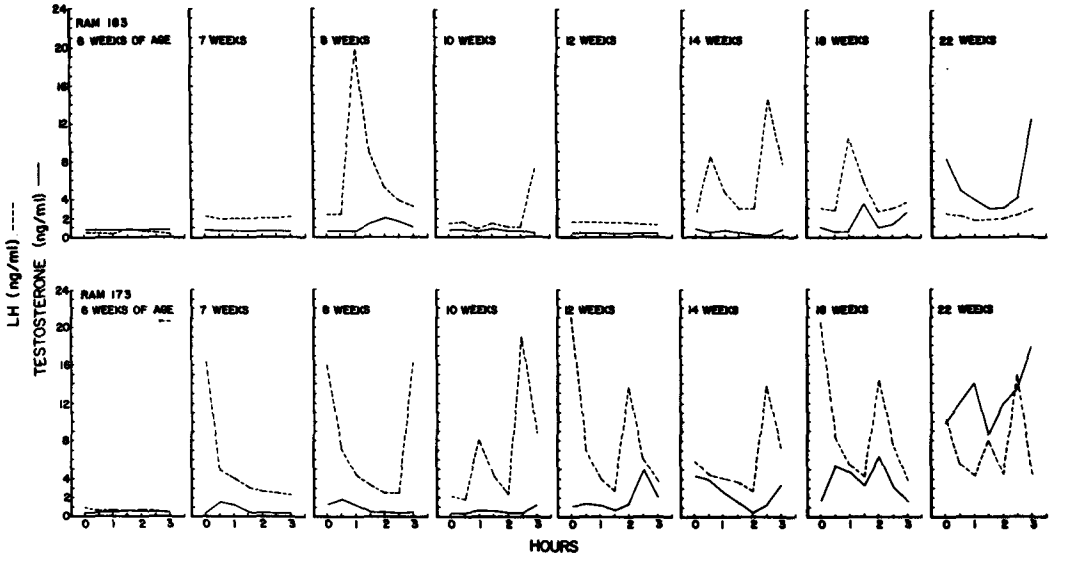

Figure 4. Patterns of systemic LH and testosterone at 6, 7, 8, 10, 12, 14, 18 and 22 wk of age for a slow (ram 183) and a rapid (ram 173) maturing Suffolk ram lamb in Group 2. Progesterone implants were present at 6 wk of age (far left panel).

= .63 (P<.01), .79 (P<.01), .33 and .32, respectively, for Suffolk rams.

Paired testes weight at 22 wk of age (table 2) did not differ (P>.05) among treatment groups, but was heavier (P<.01) for Suffolk than for Finn rams. Testes weight was correlated positively (r = .79; P<.01) with scrotal circumference at 22 wk of age within both breeds of sheep.

Seminiferous Tubule Diameter and Spermatogenesis. Seminiferous tubule diameter (table 3) increased (P<.01) with age between 14 and 22 wk of age, and was larger (P<.05) in Finn than in Suffolk rams at all ages. At 14 wk of age, seminiferous tubule diameter was related inversely to duration of the progesterone implant period in Finn rams, with the difference being significant (P<.01) between Group 1 and 4. A similar trend was found in Suffolk rams except the controls differed (P<.01) from all three progesterone-implanted groups. Because of increased testis growth after implant removal in progesterone-implanted rams, treatment differences had disappeared by 18 and 22 wk of age. Seminiferous tubule diameter growth paralleled testis growth in Suffolk rams at 14 (r = .85; P<.01), 18 (r = .70; P<.01) and 22 (r = .45; P<.05) wk of age, and in Finn rams at 14 (r = .77; P<.01) and 18 (r = .62; P<.01) wk of age, but not at 22 wk (r = .27; P>.05). Within breed correlations between mean testosterone concentration in plasma and seminiferous tubule diameter were significant (P<.01) at 14 wk of age in both Finn (r = .70) and Suffolk (r = .79) rams, but not (P>.05) at 18 (r = .33 and .32, respectively) and 22 (r = .34 and .24, respectively) wk of age. Also, seminiferous tubule diameter at 14 wk of age was correlated positively with average hormone concentration in plasma for wk 8 through 14 of age for mean LH (r = .64; P<.01), basal LH (r = .61; P<.01) and mean testosterone (r = .89; P<.01) concentrations when data for Finn and Suffolk rams in Groups 1 and 2 were combined.

One Finn ram (control) had active spermatogenesis at 14 wk of age (table 4) with 19.7% of the seminiferous tubules containing elongated spermatids. The majority of the Finn and Suffolk rams in Groups 1 and 2 had elongated spermatids in the seminiferous tubules by 18 wk of age, whereas elongated spermatids were not detected in any of the rams in Groups 3 and 4. All Finn rams produced spermatozoa by 22 wk of age compared with 70% overall for Suffolk rams.

Discussion

The slower rate of sexual maturation in Finn and Suffolk ram lambs with decreased plasma

TABLE 2. EFFECT OF PROGESTERONE IMPLANTS IN PREPUBERTAL RAM LAMBS ON SCROTAL CIRCUMFERENCE AT 10, 14, 18 AND 22 WEEKS OF AGE[a]

Breed	Group	Treatment[b]	No.	Scrotal circumference (cm) at age: 10 wk	14 wk	18 wk	22 wk	Paired testis weight at 22 wk of age, g
Finn	1	Control	5	18.5 ± 1.1[ce]	21.6 ± 1.1	26.5 ± 1.1	31.8 ± 1.1	381.6 ± 43.0
	2	4-wk implant	5	15.8 ± 1.1	19.0 ± 1.1	24.9 ± 1.1	30.1 ± 1.1	324.6 ± 43.0
	3	8-wk implant	5	14.7 ± 1.1[f]	19.7 ± 1.1	26.7 ± 1.1	31.4 ± 1.1	408.6 ± 43.0
	4	12-wk implant	5	14.1 ± 1.1[d]	18.5 ± 1.1	25.8 ± 1.1	29.9 ± 1.1	364.8 ± 43.0
	$\bar{X}$		20	15.8 ± .6[g]	19.7 ± .6[g]	26.0 ± .6	30.8 ± .6[g]	369.9 ± 21.5[g]
Suffolk	1	Control	5	19.3 ± 1.1	26.3 ± 1.1[ce]	30.1 ± 1.1	35.2 ± 1.1	507.2 ± 43.0
	2	4-wk implant	5	18.3 ± 1.1	20.2 ± 1.1[d]	25.9 ± 1.1	32.1 ± 1.1	397.3 ± 43.0
	3	8-wk implant	5	16.7 ± 1.1	21.6 ± 1.1[f]	26.0 ± 1.1	34.3 ± 1.1	524.5 ± 43.0
	4	12-wk implant	5	17.2 ± 1.1	21.2 ± 1.1[f]	25.4 ± 1.1	34.1 ± 1.1	490.5 ± 43.0
	$\bar{X}$		20	17.9 ± .6[h]	22.3 ± .6[h]	26.9 ± .6	33.9 ± .6[h]	479.9 ± 21.5[h]
Combined	1	Control	5	18.9 ± .8[c]	24.0 ± .8[ce]	28.3 ± .8	33.5 ± .8	444.4 ± 30.4
	2	4-wk implant	5	17.1 ± .8	19.6 ± .8[d]	25.4 ± .8	31.1 ± .8	360.9 ± 30.4
	3	8-wk implant	5	15.7 ± .8[d]	20.7 ± .8[f]	26.4 ± .8	32.9 ± .8	466.5 ± 30.4
	4	12-wk implant	5	15.7 ± .8[d]	19.9 ± .8[d]	25.6 ± .8	32.0 ± .8	427.7 ± 30.4

[a]Least-squares means ± SE.

[b]Treatments were the same as in table 1.

[c,d]Treatment means differ within age and breed ($P<.01$).

[e,f]Treatment means differ within age and breed ($P<.05$).

[g,h]Breed means differ ($P<.01$).

TABLE 3. EFFECT OF PROGESTERONE IMPLANTS IN PREPUBERTAL RAM LAMBS ON SEMINIFEROUS TUBULE DIAMETER AT 14, 18 AND 22 WEEKS OF AGE[a]

Breed	Treatment group[b]	No.	Tubule diameter (μm) at age: 14 wk	18 wk	22 wk
Finn	1	5	156.9 ± 8.6[c]	192.2 ± 8.6	231.6 ± 8.6[c]
	2	5	140.4 ± 8.6	182.5 ± 8.6	200.1 ± 8.6[d]
	3	5	129.3 ± 8.6	192.5 ± 8.6	222.7 ± 8.6
	4	5	116.3 ± 8.6[d]	176.0 ± 8.6	208.9 ± 8.6
	$\bar{X}$	20	135.7 ± 4.3[e]	185.8 ± 4.3[g]	215.8 ± 4.3[e]
Suffolk	1	5	158.6 ± 8.6[c]	178.0 ± 8.6	204.2 ± 8.6
	2	5	117.4 ± 8.6[d]	167.1 ± 8.6	192.0 ± 8.6
	3	5	111.0 ± 8.6[d]	168.1 ± 8.6	201.0 ± 8.6
	4	5	105.8 ± 8.6[d]	163.0 ± 8.6	209.3 ± 8.6
	$\bar{X}$	20	123.2 ± 4.3[f]	169.1 ± 4.3[h]	201.6 ± 4.3[f]
Combined	1	5	157.7 ± 6.1[c]	185.1 ± 6.1	217.9 ± 6.1[i]
	2	5	128.9 ± 6.1[d]	174.8 ± 6.1	196.0 ± 6.1[j]
	3	5	120.1 ± 6.1[d]	180.3 ± 6.1	211.8 ± 6.1
	4	5	111.1 ± 6.1[d]	169.5 ± 6.1	209.1 ± 6.1

[a]Least-squares means ± SE.

[b]Treatments were the same as in table 1.

[c,d]Treatment means differ within age and breed ($P<.01$).

[e,f]Breed means differ ($P<.01$).

[g,h]Breed means differ ($P<.05$).

[i,j]Treatment means differ ($P<.05$).

TABLE 4. EFFECT OF PROGESTERONE IMPLANTS IN PREPUBERTAL RAM LAMBS ON ONSET OF PUBERTY[a]

Breed	Treatment group[b]	Presence of spermatozoa at age: 14 wk No.[c]	14 wk %[d]	18 wk No.[c]	18 wk %[d]	22 wk No.[c]	22 wk %[d]
Finn	1	1	19.7	4	37.8 ± 7.4	5	45.1 ± 6.6
	2	0	0	4	31.1 ± 7.4	5	31.1 ± 6.6
	3	0	0	0	0	5	55.4 ± 6.6
	4	0	0	0	0	5	35.9 ± 6.6
Suffolk	1	0	0	4	37.3 ± 7.4	4	27.2 ± 7.4
	2	0	0	2	38.3 ± 10.5	3	20.7 ± 8.6
	3	0	0	0	0	2	48.6 ± 10.5
	4	0	0	0	0	5	38.1 ± 6.6
Combined	1	1	19.7	8	37.5 ± 5.2	9	37.1 ± 4.9
	2	0	0	6	34.0 ± 6.1	8	27.2 ± 5.7
	3	0	0	0	0	7	53.4 ± 5.6
	4	0	0	0	0	10	37.0 ± 4.7

[a]Rams with elongated spermatids in the seminiferous tubules were assumed to have reached puberty.

[b]Treatments were the same as in table 1 with five rams·treatment^{-1}·breed^{-1}.

[c]Number of rams in which elongated spermatids were detected in seminiferous tubules.

[d]Percentage of seminiferous tubules that contained elongated spermatids (mean ± SE).

LH and testosterone concentrations and LH pulse frequency, that occurred naturally or as a result of progesterone implants, suggests a positive functional relationship among LH secretion, testicular steroidogenesis and development, and spermatogenesis in prepubertal ram lambs. Foster et al. (1978) reported that LH pulses were evident by 1 to 2 wk of age in rapidly growing ram lambs vs 3 to 7 wk in slowly growing ram lambs, which tend to reach puberty at an older age, and that mean concentration of serum LH was determined by LH pulse frequency. Although progesterone implants decreased LH secretion, measurable LH concentrations and sporadic LH pulses were found during treatment periods and may explain why testicular growth and development occurred in progesterone-implanted rams but at a slower rate than in untreated rams or after implant removal. In ovariectomized ewes, progesterone implants reduced LH pulse frequency but not magnitude (Goodman and Karsch, 1980). Hypophysectomy of young lambs stopped growth of the testis and prevented onset of spermatogenesis; whereas, LH and follicle-stimulating hormone (FSH) treatment overcame these effects (Courot, 1967). Because FSH secretion is thought to be regulated by a nonsteroidal factor from Sertoli cells (Setchell et al., 1977), the predominant inhibitory effect of progesterone should be on LH rather than FSH secretion. However, FSH secretion was not evaluated in the present study, but progesterone treatment did not affect FSH concentration in anestrous or cyclic ewes (Saba et al., 1975).

A positive relationship between systemic LH and testosterone concentrations in prepubertal rams was indicated by lower LH and testosterone concentrations in rams implanted with progesterone than in untreated rams, and by transient increases in plasma testosterone concentrations subsequent to pulsatile LH releases as reported previously by Foster et al. (1978). Low testosterone concentrations in progesterone-implanted rams suggest either minimal conversion of exogenous progesterone to testosterone by the testes and peripheral tissue, or conversion of progesterone to other androgens as found in prepubertal male rats (Steinberger and Ficher, 1969).

Early postnatal appearance of LH pulses in ram lambs (Foster et al., 1978) and the similarity of patterns of systemic LH between intact prepubertal ram lambs and castrated adults (D'Occhio et al., 1982) would suggest minimal gonadal regulation of LH secretion in prepubertal ram lambs. Also, elevated plasma LH concentrations and pulses were maintained in the presence of increasing testosterone concentrations except at 22 wk of age (puberty) when mean LH was reduced. However, removal of one or both testes from prepubertal rams does result in increased systemic LH and FSH concentrations, but the increase after castration is delayed in prepubertal rams relative to adults (Foster et al., 1972; Land and Carr, 1975; Schanbacher, 1980). Thus, gonadal inhibition of gonadotropin secretion appears to exist in prepubertal rams but with decreased hypothalamic-pituitary sensitivity to negative gonadal feedback. This is in contrast to an absence of LH pulses and increased sensitivity to negative gonadal (estradiol) feedback found in prepubertal ewe lambs (Foster et al., 1975; Foster and Ryan, 1979). The decrease in LH at 22 wk of age (present study) may have resulted from maturation of the hypothalamic-pituitary axis and increased sensitivity to negative gonadal feedback, from the large increase in systemic testosterone concentration or from a seasonal decrease in LH secretion (Schanbacher and Lunstra, 1976). A change in sensitivity of the pituitary-testicular axis in rams during puberty was reported by Lee et al. (1976), while other studies have indicated a decrease in LH pulse frequency and mean concentration in plasma at 16 to 20 wk of age (Courot et al., 1975; Foster et al., 1978; Wilson and Lapwood, 1979). Sanford et al. (1982) found an age-associated decrease in LH from ram lambs to yearling to mature rams.

The cause of the increase in LH before removal of progesterone implants from rams in Group 4 could not be ascertained from our data. The increase may have resulted from the small decrease in systemic progesterone between 8 and 12 wk of age (figure 1) or from an escape of LH secretion from progesterone inhibition. A comparison of progesterone and LH concentrations among rams in Group 4 would favor the latter explanation.

Several studies have suggested a positive relationship among prepubertal plasma LH concentration, testis size and breed fecundity (Carr and Land, 1975; Land and Carr, 1975; Land, 1978). Alternatively, increased prepubertal LH and testosterone secretion and testis size in rams of prolific breeds may be associated with enhanced sexual development found in these breeds. Finn rams (present

study) had higher plasma testosterone concentrations (which may indicate increased LH secretion), larger seminiferous tubule diameters and reached puberty at a younger age, but contrary to other reports had smaller testes than rams of the less prolific Suffolk breed (Carr and Land, 1975; Land and Carr, 1975). Testis size was related positively to body size (r = .95 between breeds) and Suffolk rams were about 40% heavier than Finns; thus, breed differences in mature body size may qualify use of testis size as an index of physiological characteristics. Within breeds, testis size and development were related to prepubertal LH secretion.

Seminiferous tubule diameter increased with age, paralleled increases in testes size (scrotal circumference) and plasma testosterone concentration, and provided an index of spermatogenic development. Tubule diameter measurements and changes with age, and presence of spermatozoa at 18 wk in Suffolk rams were consistent with those reported by Skinner et al. (1968). The inverse relationship between duration of progesterone treatment and tubule diameter at 14 wk suggests that development of tubules before 14 wk of age was affected by length of time that LH stimulation was reduced by progesterone. Progesterone treatment delayed appearance of elongated spermatids in Groups 3 and 4, but not in Group 2, suggesting that either LH and(or) steroidal initiation of spermatogenesis occurred after 6 wk of age or suppression of LH secretion for 4 wk had no effect on maturation of the germinal epithelium. Lack of treatment effects on seminiferous tubule diameter, scrotal circumference and plasma testosterone concentration at 18 and 22 wk of age and on testes weight or spermatogenesis at 22 wk suggest that any inhibitory effects of progesterone were reversible. Results from the present study suggest that rate of sexual maturation in ram lambs is related to level of LH stimulation, which is dependent upon frequency of pulsatile LH releases, and postnatal age of the ram lamb when the LH stimulation is initiated.

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